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Light-triggered β -hairpin folding and unfolding

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A light-switchable peptide is transformed with ultrashort pulses from a β -hairpin to an unfolded hydrophobic cluster and vice versa. The structural changes are monitored by mid-IR probing. Instantaneous normal mode analysis with a Hamiltonian combining density functional theory with molecular mechanics is used to interpret the absorption transients. Illumination of the β -hairpin state triggers an unfolding reaction that visits several intermediates and reaches the unfolded state within a few nanoseconds. In this unfolding reaction to the equilibrium hydrophobic cluster conformation, the system does not meet significant barriers on the free-energy surface. The reverse folding process takes much longer because it occurs on the time scale of 30 μ s. The folded state has a defined structure, and its formation requires an extended search for the correct hydrogen-bond pattern of the β -strand.

density functional theory calculation | peptide folding | TrpZip2 | ultrafast infrared spectroscopy

Folding is a key process during the formation of a functional protein after the synthesis of its amino acid chain (1). During folding, the amino acid chains are rearranged in highly complex processes, for which a detailed understanding is still missing. Straightforward solutions of the folding problem are prevented by the high dimensionality of the protein conformational spaces and by the wide range of relevant time scales. Thus, for complexity reduction, one has to focus on typical protein substructures, which are small enough to be analyzed by present-day technology. With corresponding peptide model systems there is a chance to monitor the formation of secondary structures, to identify characteristic intermediate states of these folding dynamics, and to carry out realistic simulations on a molecular level. An interesting class of model systems are light-triggered peptides, within which the incorporation of a photoresponsive element can initiate structural changes.

Among corresponding photoresponsive chromophores, azobenzene derivatives have become a popular choice when it comes to selecting a fast conformational trigger for peptide refolding, because their structure significantly changes on time scales of a few hundred femtoseconds after photoexcitation. Indeed, an azobenzene chromophore, used as a backbone element within cyclic peptides, was shown to induce large-scale conformational changes, whose dynamics could be monitored by visible (2) and IR (3) spectroscopy. Moreover, the experimental results clearly showed that the initial, strongly driven conformational changes of the peptide proceeded within a few picoseconds after the trigger event. Externally linked to an α -helix, azobenzene also was used for unfolding (4) and refolding (5) of an α -helical model peptide in the nanosecond to microsecond time range.

A prominent secondary structure motif is the β -sheet, for which a β -hairpin represents a minimal model (6, 7). We and others (8–10) have recently focused on the design of photocontrolled β -hairpin peptides. To enable an ultrafast initiation of β -hairpin folding and unfolding the azobenzene derivative 3-(3-aminomethylphenylazo)phenylacetic acid (AMPP) was used as a reversible light switch (8, 10), which, in the cis isomeric state, acts as β -turn mimetic (see Fig. 1A). Indeed, as shown by NMR, the peptide folds into β -hairpin conformations as long as the azo-

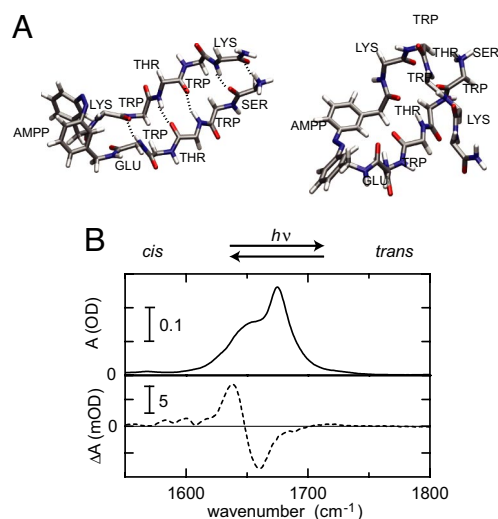


Fig. 1. Cis and trans conformations of the light-switchable peptide. (A) (Left) NMR-derived sample structure (10) of the β -hairpin peptide with the AMPP-chromophore in the cis conformation. (Right) The cis-to-trans photoisomerization of the chromophore drives the peptide into a different equilibrium conformational ensemble, for which a representative structure is shown. (B) IR absorption spectrum of the β -hairpin model peptide in the trans form (solid curve) and light-induced absorption difference observed upon illumination around 370 nm, leading to the formation of the cis form (broken curve).

benzene switch is in its cis form (10). This conformation is stabilized by hydrogen bonds between the two peptide β -strands and by hydrophobic interactions between aromatic residues incorporated into the two peptide strands. Upon cis-to-trans isomerization, the geometry of the switch prevents a likewise tight coupling between the N- and C-terminal parts of the peptide, and the peptide unfolds into an ensemble of globular structures, which are stabilized apparently by hydrophobic forces. Therefore we call the trans structures of the peptide “hydrophobic clusters.”

In this paper we present results obtained with this β -hairpin model by ultrafast UV pump, IR probe spectroscopy combined

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Abbreviations: AMPP, 3-(3-aminomethylphenylazo)phenylacetic acid; DFT, density functional theory; INMA, instantaneous normal mode analysis; MD, molecular dynamics; MM, molecular mechanics.

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with instantaneous normal mode analysis (INMA) and molecular dynamics (MD) simulations, which yielded detailed information not only on the kinetics but also on the structural intermediates of the light-triggered folding and unfolding processes.

Stationary Spectroscopy of the Model Peptide. Our IR experiments study the β -hairpin model dissolved in deuterated methanol. They focus on the spectral region of the amide I band generated predominantly by the C=O stretch vibrations of the backbone carbonyl groups. The amide I band is known to code the secondary structures of peptides and proteins (11, 12). A structural decoding of the absorption spectrum in the amide I region has to rely on empirical rules, because reliable theoretical approaches for interpretation of band shapes and peak positions in terms of protein structures are still lacking, although intense efforts are undertaken for improvement (13, 14). The empirical rules are based on experimental observations of model compounds (e.g., *N*-methylacetamide) and proteins (11, 12). For *N*-methylacetamide in a solvent with a low dielectric constant, the C=O group vibrates at high frequencies $\nu/c \approx 1,680$ – $1,700$ cm^{-1} (14, 15). The vibrational frequency is lowered with rising polarity of the surroundings (14, 15) such that, e.g., in methanol, which is the mildly polar solvent used in our experiments, the C=O frequency is found at $\approx 1,660$ cm^{-1} . In water, which is a much more polar and protic solvent than methanol, the vibrational frequency is further reduced to $\approx 1,625$ cm^{-1} (14, 15). Additional changes of the spectral positions are found in proteins with dominant secondary structure elements. Here, the spectral positions are related to the structural arrangement of the polypeptide chains and the correspondingly shaped electrostatic and transition dipole interactions (11, 12).

The IR absorption spectrum of the model peptide in deuterated methanol with the azobenzene chromophore in the trans form is drawn in Fig. 1*B* (solid curve). This spectrum features a strong peak related to the amide I mode at $1,675$ cm^{-1} . A shoulder extends to lower frequencies down to the $1,620$ – cm^{-1} range. Illumination of the sample around 370 nm leads to the formation of *cis*-azobenzene and causes changes in the IR spectrum (see Fig. 1*B*, dashed curve). There is a pronounced increase in the IR absorption at $1,640$ cm^{-1} , whereas some decrease is found at $1,660$ cm^{-1} . Two weak bands of increased absorption at $1,583$ and $1,600$ cm^{-1} are related to the azobenzene chromophore in the *cis* form. Assuming for a preliminary interpretation of the absorbance properties that the amide I band shape of our peptide model is mainly steered by local hydrogen bonding interactions and by the dielectric shielding of the amide dipoles caused by the mildly polar solvent (and thus neglecting the more complicated transition dipole interactions and corresponding secondary structure effects) yields the following picture, which agrees well with the NMR structural data (10). In the trans peptide, only few amide groups exhibit significant intramolecular hydrogen bonding, as is indicated by the weakness of the low-frequency shoulder at $1,640$ cm^{-1} . The pronounced higher-frequency peak at $1,675$ cm^{-1} suggests that most amide dipoles are only weakly shielded by the mildly polar solvent methanol (note here that, in water, one would expect a different behavior because of its larger polarity). Upon formation of the *cis* isomer of the azobenzene chromophore, the attached model peptide is transformed to the β -hairpin-like structure with an increased amount of strong intramolecular hydrogen bonds (10). This NMR result nicely fits the observed absorption changes featuring a pronounced absorption increase at $1,640$ cm^{-1} (see Fig. 1*B*, dashed curve).

Computational Description of the Amide I Spectral Changes. For the given, rather small, peptide model, high-level theoretical descriptions can be applied to check the above structural interpretation of the observed spectral changes. For this purpose, the

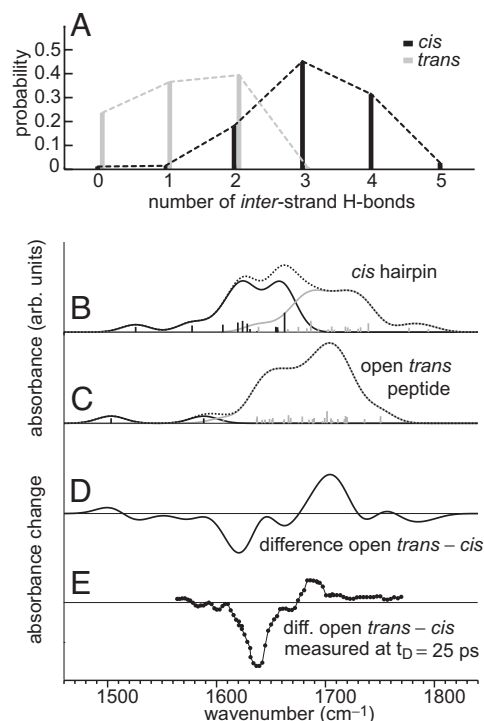


Fig. 2. Description of trans-cis difference spectra. (A) Probability distributions of inter- β -strand hydrogen bonds for the *cis* (black dashed line) and *trans* (gray dashed line) equilibrium ensembles at 300 K derived from replica exchange MD simulations (see *Materials and Methods*). (B–D) Amide I bands of the model peptide calculated by DFT/MM using the INMA technique; smooth bands are obtained from line spectra by Gaussian convolution (width, 13 cm^{-1}). (B) INMA spectrum derived from three *cis*-hairpin snapshots. Black lines represent bands of C=O groups with inter- β -strand hydrogen bonds; gray lines represent bands of C=O groups interacting with the solvent or with amino acid side groups or showing no hydrogen bonding. (C) INMA spectrum of three selected open *trans* structures. (D) Difference between the *trans* and *cis* structures. (E) Transient spectrum at $t_D = 25$ ps taken from Fig. 3*B*.

β -hairpin unfolding in the given solvent (deuterated methanol) was first simulated by MD, and, subsequently, the amide I bands both before and after photoisomerization were computed by a hybrid approach combining density functional theory (DFT) with a molecular mechanics (MM) approach [for details see *Materials and Methods* and [supporting information \(SI\) Materials and Methods](#)]. Because these DFT/MM calculations require an enormous computational effort, amide I bands were calculated only for three samples of *cis* and *trans* peptide structures. These sample structures were carefully selected from large equilibrium ensembles of solvated peptide structures, which were generated by extended MD simulations of peptide-solvent systems as described in *Materials and Methods*. Our selection of *cis* and *trans* peptide sample structures was guided by the desire to learn how the amide I bands change when inter- β -strand hydrogen bonds originally present in a nicely ordered *cis*- β -hairpin structure become disrupted upon *cis*-*trans* isomerization of the azobenzene switch. This approach is supported by results of replica exchange solute tempering simulations (16) on the 300 K equilibrium conformational ensembles of the *cis*- and *trans*-hairpin, which show that the *cis* conformations exhibit a much larger number of inter- β -strand hydrogen bonds than the *trans* states (see Fig. 2*A*). For DFT/MM, we therefore selected from the two MD equilibrium ensembles *cis*-hairpin structures featuring a maximum number of interstrand hydrogen bonds and *trans* structures, in which all these hydrogen bonds were broken. Because the selected structures do not represent the *cis* and *trans*

equilibrium ensembles in any statistically relevant sense, the computed absolute amide I spectra do not aim at an explanation of observed stationary spectra. Instead, the difference of the computed cis and trans spectra serves to predict the changes, which are to be expected from the breaking of inter- β -strand hydrogen bonds in a cis-trans transition.

Fig. 2*B–D* presents our DFT/MM results for the amide I bands of the selected cis (Fig. 2*B*) and trans (Fig. 2*C*) structures as well as for the trans-cis difference (Fig. 2*D*) predicting the spectral effect that is expected to result from the disruption of all inter- β -strand hydrogen bonds. The DFT/MM spectrum of the cis-hairpins in Fig. 2*B* demonstrates that amide I bands of C=O groups involved in inter- β -strand hydrogen bonds (black lines) are generally shifted toward frequencies that are lower than those of C=O groups interacting with the solvent or with side chains (gray lines). This classification was possible because most of the calculated amide I normal modes were found to be localized at one or two C=O groups. Because the number of strong intrapeptide hydrogen bonds is much smaller in the trans structures than in the cis structures [there are no interstrand hydrogen bonds and only two accidental intrastrand hydrogen bonds (black line)], the computed trans spectrum (Fig. 2*C*) is blue-shifted. The blue-shift is evidenced by the computed trans-cis difference spectrum of Fig. 2*D*. Thus, the shown DFT/MM results strongly support the preliminary interpretation given further above for the stationary cis-trans difference spectrum in Fig. 1 and will be important for the structural decoding of time-resolved difference spectra, to which we turn now.

The cis-to-trans Unfolding Reaction. For the time-resolved experiments, the unfolding process was initiated by illuminating the AMPP chromophore of the β -hairpin model with short light pulses (duration 700 fs) at $\lambda = 404$ nm (17). Changes in the IR-absorption spectrum induced by the exciting light pulse were recorded by properly delayed broadband IR pulses (duration 100 fs). Because the experiment measures the time dependences in the form of difference spectra recorded as a function of time delay, we can use two representations of the data. In SI Fig. 6 we focus on the time dependence by showing a set of absorption data recorded as a function of time delay t_D at characteristic frequency positions. Here, we show in Fig. 3 a series of light-induced absorption difference spectra (red lines) recorded at various delay times after photoexcitation. In the depicted range of the amide I band, these spectra encode the differences between the transient trans peptide structures and the original cis structure. Fig. 3*A Right–D Right* present backbone snapshots that were taken from a representative MD simulation of the photoinduced unfolding at the corresponding delay times. The transient spectra in Fig. 3 show the signatures of hydrogen-bond breaking discussed above. Fig. 3*A Left* provides information for a delay time $t_D = 1.6$ ps, when the AMPP chromophore has already acquired the new trans isomeric state. The pronounced absorption decrease peaking at $1,645\text{ cm}^{-1}$ and the weakly enhanced absorption at $1,690\text{ cm}^{-1}$ resemble those of the calculated spectrum (Fig. 2*D*), thus indicating disruption or weakening of hydrogen bonds and the exposure of initially hydrogen-bonded carbonyls to the solvent. Additional features of the 1.6-ps spectrum, i.e., the broadband absorption increase and decrease at the positions of the original bands of the cis ensemble at $1,670\text{ cm}^{-1}$ (amide I) and $1,603$ and $1,585\text{ cm}^{-1}$ (AMPP) point to an elevated temperature of the molecule, as expected after isomerization of the excited chromophore and its internal conversion to the ground state (18). In the electronic ground state, most of the excitation energy is stored in the molecule in the form of vibrational excitation, which causes the observed absorption change. Subsequently, the excess vibrational excitation relaxes on the few-picosecond time scale. The 1.6-ps structural snapshot from a MD simulation of the light-induced hairpin unzipping (Fig. 3*A Right*) fits nicely to this interpretation. Compared with the initial cis-azobenzene- β -hairpin (brown structure), the chromophore is in

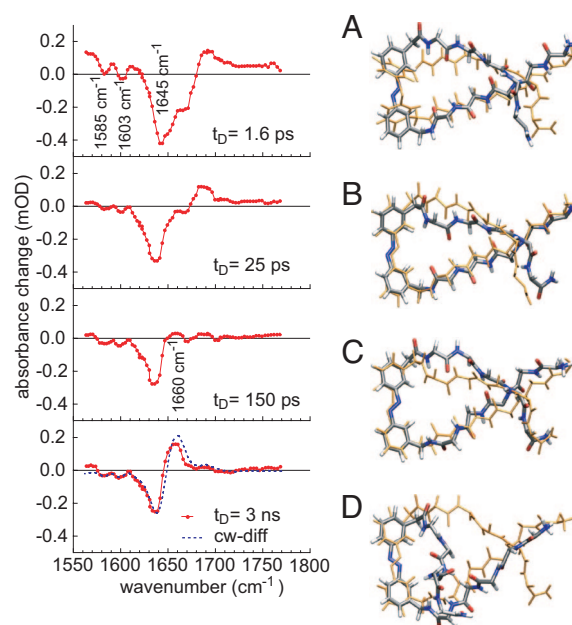


Fig. 3. Transient IR spectra and corresponding peptide structures. (*Left*) Transient absorption differences recorded at delay times of 1.6 ps (*A*), 25 ps (*B*) 150 ps (*C*), and 3 ns (*D*). The dotted curve in *D* is the steady-state difference spectrum calculated by subtraction of the IR spectrum of the trans ensemble from that of the cis- β -hairpin. (*Right*) Snapshots (blue and gray) of the hairpin unzipping taken from a MD simulation (see SI Materials and Methods) at the same delay times as in *Left*. Respective precursor structures are drawn in brown. In the drawing at the top, the brown precursor is the initial cis structure.

trans conformation and the hairpin strands close to the chromophore are widened when the initial hydrogen bonds are broken. According to the simulations, a significant amount of heat remains within the peptide at this moment.

In the IR transient spectrum recorded at $t_D = 25$ ps (see Fig. 3*B*, solid trace), there are strong indications for additional breakings of interstrand hydrogen bonds (absorption decrease shifted from $1,645\text{ cm}^{-1}$ to $1,637\text{ cm}^{-1}$) in a meanwhile thermally equilibrated peptide, which is reflected in the recovery of the broad absorption increase found at early times. The absorption increase visible around $1,690\text{ cm}^{-1}$ indicates that a number of amide groups remains in surroundings of low polarity. Apparently, at $t_D = 25$ ps, the molecule has reached a structure with a minimum number of interstrand hydrogen bonds. The MD simulation [see snapshots belonging to 25 ps and 1.6 ps (brown structures), respectively, in Fig. 3*B*] supports the interpretation that most of the interactions between the two strands have vanished at that time. The difference spectrum at $t_D = 25$ ps resembles well the calculated trans-cis difference spectrum (see Fig. 2), apart from an expected overestimate of band widths inherent to the INMA approach (19, 20).

During the following period of ≈ 100 ps (see Fig. 3*C* and *D*), IR intensity is shifted from the $1,690\text{-cm}^{-1}$ range toward lower frequencies, pointing to a resolution of the carbonyl groups. The transient spectrum at 150 ps exhibits solely a negative band ($1,635\text{ cm}^{-1}$). At this moment, the two strands of the original β -sheet are largely separated, the resolution of the carbonyl groups by the solvent is complete, but the final arrangement of the trans form of the peptide is not yet reached. The incomplete relaxation is documented by the fact that the 150-ps transient absorption spectrum lacks any increased absorption near $1,660\text{ cm}^{-1}$, where the stationary difference spectrum (dashed lines in Figs. 3*D* and 1*B*) shows a pronounced peak. According to the MD simulations (Fig. 3*D Right*), the conformational relaxation dynamics toward the ensemble of NMR trans structures (10)

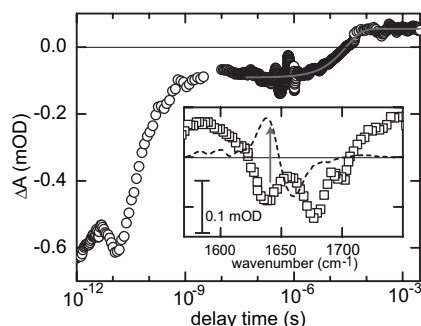


Fig. 4. Light-induced transient absorption changes recorded at $1,641\text{ cm}^{-1}$ after excitation of the trans form of the model peptide at 404 nm ($t < 4\text{ ns}$) and 355 nm ($t > 10\text{ ns}$) for triggering the folding reaction. (Inset) Absorption difference spectrum recorded at a time of 3 ns (symbols) and stationary trans-cis difference spectrum (dashed curve). The arrow marks the observation wavenumber of $1,641\text{ cm}^{-1}$.

occurs on a time scale of a few nanoseconds. Correspondingly, the positive band observed at $1,660\text{ cm}^{-1}$ appears with a time constant of 700 ps (Fig. 3B) and signifies the approach of the peptide toward the *trans*-azobenzene equilibrium ensemble. At the end of our observation time window, at 3 ns (see Fig. 3D), the transient absorption difference spectrum (Fig. 3D, solid curve) is very similar to the stationary difference spectrum (Fig. 3D, dashed curve), apart from a small 3-cm^{-1} frequency shift. Apparently, the peptide has nearly completed its way to the *trans*-azobenzene conformational ensemble within this very short time.

The trans-to-cis Folding Reaction. To analyze the reverse process, i.e., the refolding of the β -hairpin, transient IR spectra were recorded after illumination of the *trans*-azobenzene ensemble. A pronounced initial absorption decrease was observed around $1,650\text{ cm}^{-1}$ (data not shown), which rapidly decays on the time scale of 10 ps (see Fig. 4, data recorded at $1,641\text{ cm}^{-1}$). After several nanoseconds, an absorption difference spectrum is found (compare with Fig. 4 Inset, symbols), which exhibits very small amplitudes and a shape quite different from that of the stationary trans-to-cis difference spectrum (Fig. 4 Inset, broken curve). The small amplitudes of the difference spectrum indicate that a state has been reached that resembles the initial *trans*-azobenzene peptide, i.e., has the character of a hydrophobic cluster. The subsequent absorption changes from 10 ns onward have been recorded at a single probing frequency of $1,641\text{ cm}^{-1}$. After some artifacts due to pump-induced density fluctuations in the sample (21) on the time scale of 10^{-7} s , a $30\text{-}\mu\text{s}$ time constant results from the transient absorption data. This transient leads from a state with reduced absorption found on the nanosecond time scale to a weak absorption increase. The data demonstrate that, in the trans-to-cis folding process, the stationary absorption is eventually reached with a time constant of $30\text{ }\mu\text{s}$. Thus, the intermediate state formed after photoisomerization and initial relaxation folds into a β -hairpin on that time scale.

It is interesting to compare the observed folding time constant of $30\text{ }\mu\text{s}$ with results obtained with other experimental techniques. Transient temperature jump experiments in water or water guanidine hydrochloride solutions have been reported in the literature for the TrpZip2 β -hairpin peptide, which contains the same sequence of the two strands as our model peptide (22, 23). Detection-dependent kinetics in a range below $10\text{ }\mu\text{s}$ have been reported for TrpZip2 with fluorescence techniques that probe the hydrophobic clusters, i.e., the Trp side chains (23). Heterogeneous folding was found with stationary techniques pointing to a rough energy landscape of the unfolded peptide (24). Folding times in the $2.5\text{-}\mu\text{s}$ range are reported from a model-based analysis of transient IR data (probing the backbone

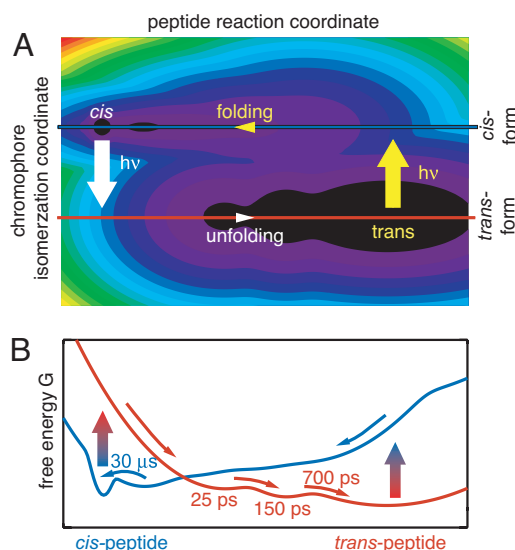


Fig. 5. Free energy surfaces. (A) Two-dimensional representation of a schematic free-energy surface G plotted as a function of the isomerization coordinate of the chromophore (vertical axis) and the reaction coordinates of the cis and trans conformations of the peptide strands (horizontal axis). Light absorption (arrows) in the chromophore drives the system toward the new conformational ensemble at the bottom of the free-energy curves. (B) Horizontal cuts through the free-energy surface along the lines in A. The blue curve pertains to the cis isomeric form of the switch. The narrow minimum on the left represents the folded cis structures, and the adjacent broad minimum accounts for the unfolded cis ensemble. The red curve relates to the trans state of the switch and serves to illustrate the relaxations after the cis-to-trans photoisomerization.

carbonyls) recorded after the application of a short heating pulse (22). Apparently the temperature rise of $\approx 10\text{--}15\text{ K}$ induced in these temperature-jump experiments causes a distortion of the folded peptide structure (25) that seems to be much weaker than the strong structural changes induced by the optical triggering process of the present β -hairpin model peptide. Thus the subsequent refolding process in the temperature-jump experiment requires a search only within a limited conformational space.

Model Scheme for the Light-Triggered Reaction. To visualize the experimentally observed light-induced structural dynamics of the β -hairpin peptide, we employ the schematic free-energy surface drawn in Fig. 5. In the unfolding process, light absorption and isomerization of the azobenzene transfers the cis equilibrium structures from the corresponding minima of the free-energy surface to an elevated and steep part of the free-energy surface belonging to the trans isomeric form (Fig. 5, white arrow). Here, a large mean force drives relaxations and structural changes, which lead on the time scale of 25 ps to modified peptide structures with broken native hydrogen bonds. From that moment onward, the peptide structures relax toward the trans equilibrium ensemble of the azobenzene peptide. The resolution of the carbonyl groups within their new surroundings is completed within 150 ps . With a time constant of 700 ps , further rearrangements lead to a structural ensemble that closely resembles the *trans*-azobenzene state. In Fig. 5 the variety of structural subfamilies belonging to the trans isomer is schematically reflected by the smooth and wide minimum of the free-energy surface. In the light-triggered refolding reaction (yellow arrow), the peptide also is transferred to a steep region of the free-energy surface belonging to the cis state (Fig. 5, blue line). Fast relaxational motions guide the structural ensemble into a long-lived intermediate state characterized by a shallow local minimum. Microseconds are required to finally reach the or-

dered hydrogen-bond pattern of the cis-hairpin ensemble indicated in Fig. 5B by the narrow minimum of the blue curve.

We would like to stress the following key results of our investigation on folding and unfolding of the model peptide. (i) We have provided a structurally and temporally resolved monitoring for the complete processes of β -hairpin folding and unfolding. The observations reveal ultrafast dynamics with several intermediates on the subnanosecond time scale. The observed light-induced processes comprise, e.g., the subnanosecond unfolding into a hydrophobic cluster and the rearrangement of one such cluster into another that is finished on the time scale of a few nanoseconds. (ii) In our model peptide, the AMPP photoswitch is converted by light into a thermally stable β -turn within a few picoseconds. Corresponding reactions of local loop formation take nanoseconds in freely fluctuating peptides (26, 27). Both reactions are therefore much faster than the 30 μ s, which, according to our data, are required for the transition from a hydrophobic cluster containing the correct β -turn into an ordered hairpin structure. Thus, this transition appears to be the rate-limiting step of β -hairpin folding (compare with the discussions in refs. 28 and 29).

Materials and Methods

Sample Preparation. The β -hairpin sample with the amino acid sequence H-Ser-Trp-Thr-Trp-Glu-AMPP-Lys-Trp-Thr-Trp-Lys-NH₂ was synthesized as described in ref. 10. The sample was dissolved in deuterated methanol (methanol-*d*₄) (Merck, Darmstadt, Germany) to yield a concentration of 1.8 mM. The sample was held in a closed flow cycle, allowing for complete sample exchange between subsequent laser shots. The sample thickness was 220 μ m.

Stationary IR Spectroscopy. Stationary IR absorption and difference spectra were recorded with an IFS66 FTIR spectrometer (resolution, 2 cm^{-1} ; Bruker, Ettlingen, Germany). The isomerization state of the azobenzene chromophore of the hairpin peptide was prepared by illumination of the sample via optical fibers with light at appropriate wavelengths. Switching from cis to trans was performed by light at wavelengths obtained from a KLC 2500 light source equipped with a 3-mm GG 400 filter (Schott, Mainz, Germany). For the trans-to-cis photoconversion, light from a HgXe arc lamp (LOT, Darmstadt, Germany) equipped with UG 1 and WG 320 glass filters (Schott) was used. The illumination procedure allowed us to populate the cis state to 90%. Continuous illumination of the sample with appropriate light was used to maintain the required photo-stationary state during the femtosecond time scale experiments.

Femtosecond IR Spectroscopy. The time-resolved experiments were performed in the visible pump, IR probe scheme. Details of the experimental set-up have been published (17). The most important features are as follows. The folding dynamics was initiated by a laser pulse centered at 404 nm with an energy of 4.4 μ J and with a pulse duration of 700 fs [spot size in the sample cell, 190 μ m (FWHM)]. The IR probe pulse was generated as previously described (17) by optical parametric amplification and difference frequency generation. The probe pulses were centered at 1,670 cm^{-1} , with a spectral bandwidth of 130 cm^{-1} [spot size in the sample cell, 95 μ m

(FWHM)]. The transient absorption change was recorded with a spectral resolution of 2.5 cm^{-1} by a 32-channel mercury-cadmium-telluride array. The plotted absorption data represent magic angle polarization between pump and probe pulses.

Microsecond IR Spectroscopy. For the experiments on the microsecond time scale (see Fig. 4 *Inset*), the third harmonic of a nanosecond neodymium-doped yttrium-aluminum-garnet laser was used (355 nm) as an excitation pulse (duration, <8 ns). A single-channel IR detector recorded the transmission change of the light from a continuous IR probe laser at 1,641 cm^{-1} . For details of the set-up, see ref. 30.

Computational Methods. The β -hairpin unfolding was first simulated by MD, and, subsequently, the amide I bands both before and after isomerization were computed by a hybrid approach combining DFT with a MM approach. Details of the used MD and DFT/MM methods are documented in *SI Materials and Methods*.

In brief, a periodic orthorhombic dodecahedron (inner radius, 25 Å) was filled with rigid MM methanol-*d*₄ models (31). The system was equilibrated by MD for 1 ns in the *NpT* ensemble using the program EGO-MMII (32). Temperature ($T = 300$ K) and pressure ($p = 1$ bar; 1 bar = 100 kPa) were controlled by Berendsen thermostats and barostats (33). Subsequently, the 10 best refined NMR structures (10) of the cis-AMPP hairpin were solvated by removing all overlapping methanol models followed by a 250-ps *NpT* equilibration. For the peptide, the CHARMM22 force field (34) was chosen, supplemented by DFT-derived parameters for the AMPP dye (35). Hydrogen bonds were kept rigid by M-SHAKE (36). The ultrafast (≈ 250 fs), light-induced, cis-trans isomerization of the AMPP dye by MM-MD simulation was enabled by an additional model potential (2, 35). Intermediate structures obtained 100 ps after isomerization were selected as representatives for the model peptide on its way to the equilibrium ensemble of the trans conformations.

The IR bands of the cis and open trans peptide in the amide I spectral range were derived from DFT/MM calculations (37) by the protocol for an INMA given in refs. 19 and 20. For the cis case, we selected three snapshots with a maximal number of inter- β -strand hydrogen bonds, and, for the trans case, we selected three snapshots without such hydrogen bonds from the equilibrated NMR structures. The peptide backbone building up the DFT fragment was treated with the plane-wave DFT code CPMD (38). The covalent linkages between the peptide backbone and MM fragment (dye, side chains, and solvent) were treated as described in ref. 37.

The INMA procedure yielded for each of the snapshots the vibrational frequencies and IR intensities of all backbone normal modes of the deuterated peptide. IR spectra of the backbone C=O stretching modes of the peptide were constructed by Gaussian smoothing of the obtained line spectra.

Additionally, two replica exchange solute tempering simulations (duration, 5 ns) (16) were performed for the cis and the trans isomers of the β -hairpin model to estimate the numbers of inter- β -strand hydrogen bonds of the equilibrium ensembles.

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